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Sensitive and Selective Quantitation of N-Nitroso Dabigatran Etexilate Impurity in Dabigatran Etexilate API using the Agilent 6495C LC/TQ

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Introduction

Nitrosamines are of concern as most of them have been reported to be potent mutagens in rodents and are potential carcinogens. Therefore, it is important to control these impurities at or below the stipulated specification limits. API-derived complex nitrosamines are known as nitrosamine drug substance-related impurities (NDSRIs). Multiple recalls of pharmaceutical drug products due to the presence of NDSRIs have drawn the attention of regulators and manufacturers. By considering the complexity of the global pharmaceutical supply chain, manufacturers must be more diligent to protect consumers by screening their APIs and drug products for the presence of NDSRIs. NDSRIs can be formed from both secondary and tertiary amines. Secondary amines can easily undergo nitrosation in the presence of trace amounts of acid. In the case of tertiary amines, this reaction is possible due to nitrosative cleavage or dealkylation. Dabigatran etexilate is a prodrug of dabigatran (for most NDSRIs, analytical methods are not yet available, so manufacturers will need to develop, qualify, and validate a method that is suitable for the sample matrix and specific for the nitrosamines in question).

The analytical method developed must be sensitive enough to quantify the nitrosamines of interest down to a level that corresponds to 10% of specification limit.

In this poster we highlight a highly specific, sensitive, and reproducible method that was developed for the quantitation of N-nitroso dabigatran etexilate impurity in dabigatran etexilate mesylate drug substance using the 6495C triple quadrupole LC/MS (LC/TQ) coupled to the 1290 Infinity II LC.

Challenges in NDSRI Analysis and Tailored Solutions

High Sensitivity:
Challenge: Achieving target limit of quantitation (LOQ)
Solution: Agilent Jet Stream (AJS) ESI is the preferred ion source with positive ionization mode. Inclusion of buffer additives to improve sensitivity

Improve API Stability:
Challenge: Hydrolytic and pH dependent degradation
Solution: Selection of organic diluent and pH adjustment to improve API stability

High Recovery:
Challenge: Unable to meet lower level (LOQ); high matrix interferences
Solution: Achieved recovery within acceptance limits with sample extraction optimization and chromatography

Reduce Carry Over:
Challenge: Carry over is observed for the analyte specific to column
Solution: Minimized by optimizing the LC Gradient and optimized organic mobile phase

Experimental

LC Configurations and Parameters:

Chromatography method conditions, MRM and source parameters were optimized, and setup as describe in table 1, 2 and 3, respectively.

Table 1. LC configurations and parameters

Parameters	Value		
Instrument	Agilent 6495C LC/TQ coupled to 1290 Infinity II LC		
Needle Wash	S1: MeOH: Water (80/20); S2: Water: MeOH (80/20)		
Sample Diluent	MeOH: ACN (90:10, v/v) with 0.05% NH_4OH (of strength 25%)		
Multisampler Temperature	10 °C		
Injection Volume	7 µL		
Analytical Column	Agilent Infinity Lab Poroshell HPH-C18, 150 × 3.0 mm, 2.7 µm, p/n 693975-502 T		
Column Temperature	40 °C		
Mobile Phase A	1 mM ammonium trifluoroacetate with formic acid in water		
Mobile Phase B	MeOH: ACN		
Flow Rate	0.5 mL/min		
Run Time	20 min		
Gradient	Time (min)	A (%)	B (%)
	0	55	45
	3	55	45
	6	40	60
	13	40	60
	15	15	85
	17	15	85
	17.1	55	45
	20	55	45

Table 2. Compound related parameters

Precursor Ion (m/z)	Product Ion (m/z)	Dwell (ms)	Fragmentor (V)	CAV	CE	Polarity
657.3	364.1	100	166	4	16	Positive
657.3	433.2	100	166	4	24	Positive
657.3	627.3	100	166	4	20	Positive

Table 3. Source parameters optimized for selectivity and sensitivity

Source/Gas Parameters	Value
Gas Flow	12 L/min
Gas Temperature	250 °C
Sheath Gas Flow	12 L/min
Sheath Gas Temperature	400 °C
High-Pressure Radio Frequency	150
Polarity	Positive
Nozzle Voltage	2000 V
Capillary Voltage	3200 V
Nebulizer Pressure	35 psi
Low-Pressure Radio Frequency	60

Results and Discussion

Linearity, Sensitivity at Limit of Detection and Limit of Quantitation. Critical method development parameters like sensitivity, linearity, specificity, reproducibility, and recovery, were established. Sensitivity parameters, LOD and LOQ were established, as defined by ICH guidelines, where the S/N values were 3.3 and 10 for the LOD and LOQ, respectively. Calibration curves constructed for N-nitroso dabigatran were found to be linear within the 0.01 to 1.0 ng/mL (0.002 to 0.2 ppm with respect to API) concentration range. The value of R2 was 0.9993 for the equation y = mx + c, where m is the slope and c are the intercept with 1/x2 as weighting factor.

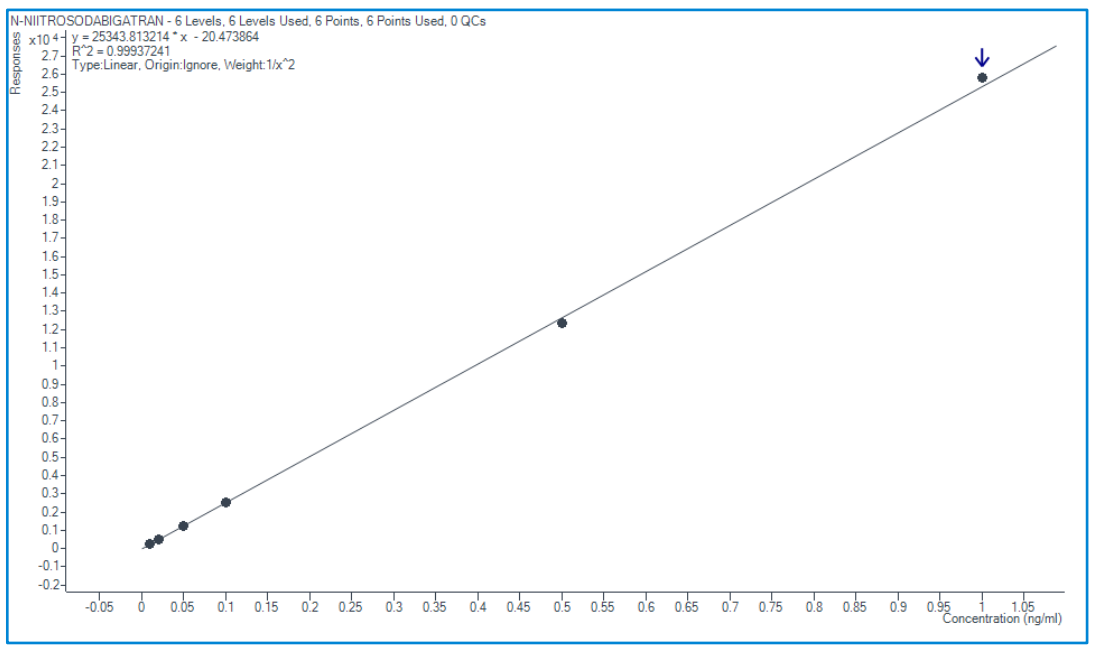

Figure 1. Linearity from 0.01 ng/mL to 1 ng/mL

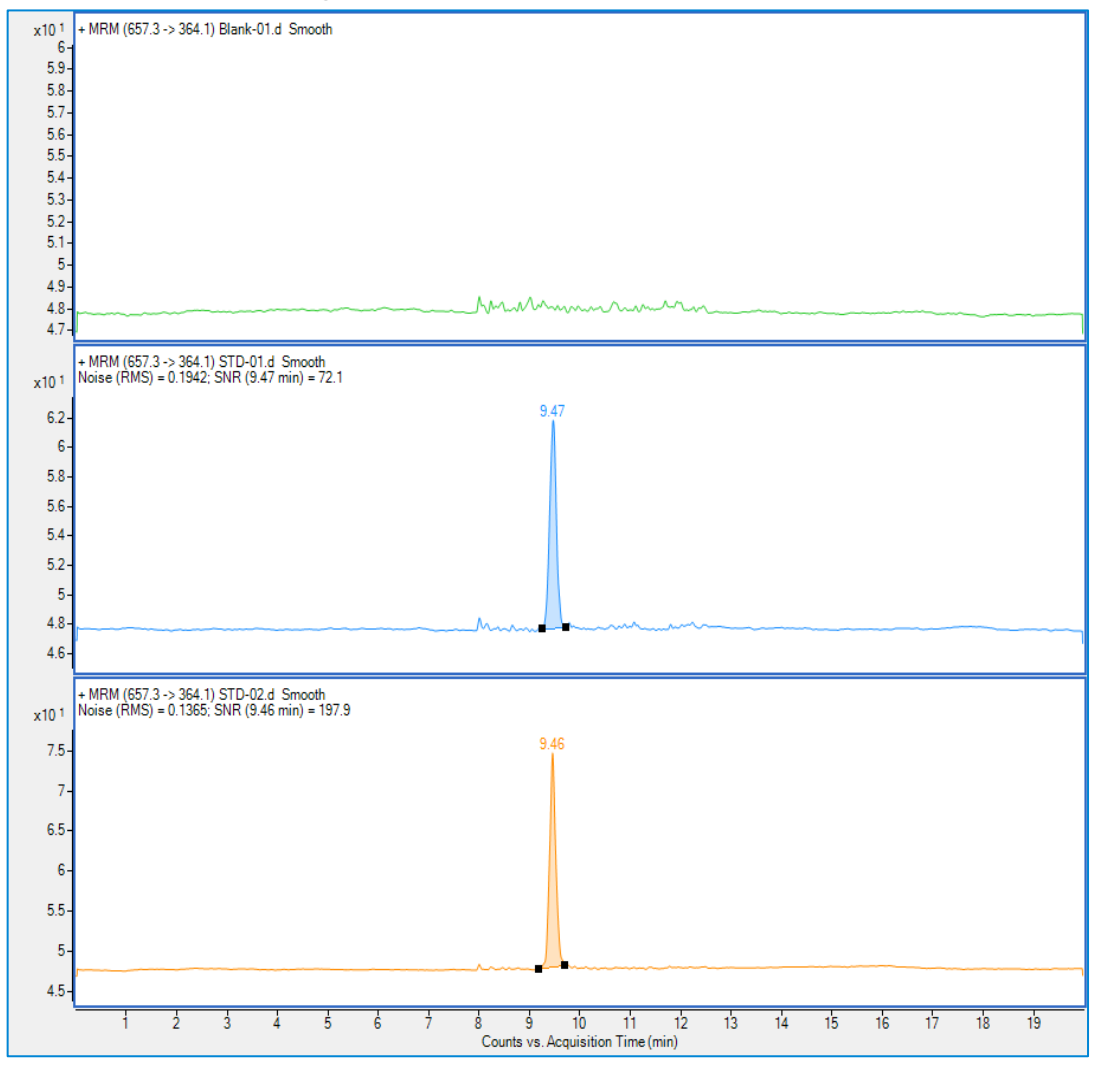

Figure 2. LOD 0.006 ng/mL (0.0012 ppm) and LOQ 0.01 ng/mL (0.002 ppm) with S/N > 70:1 and S/N > 190:1 respectively, calculated using RMS.

Method Chromatography Separation, Selectivity and Specificity.

The method conditions were optimized to provide sufficient selectivity and sensitivity and achieve better resolution between the impurity and the API. Reversed-phase chromatography was performed using the Agilent Poroshell HPH-C18 column (3 × 150 mm, 2.7 µm), with a mobile phase consisting of ammonium trifluoroacetate and formic acid in water as aqueous phase, and a mixture of MeOH and ACN (80:20, v/v) as organic phase.

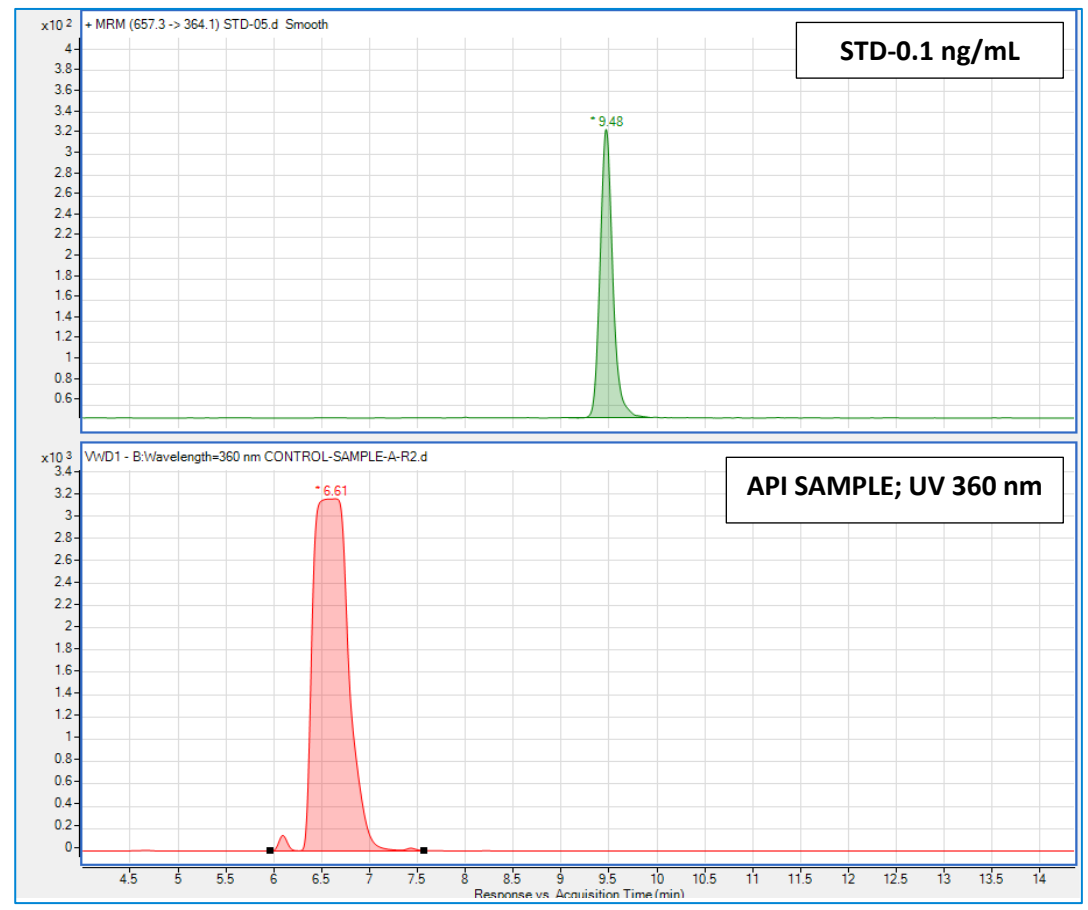

Figure 3. Separation between impurity and API

Specificity in Sample Matrix is Studied and Evaluated with the Precise Selection of Quantifier and Qualifier Ion

MRM chromatograms are acquired for the standard at 0.1 ng/mL and control sample to select the quantifier ion specific to analyte and free from matrix interference.

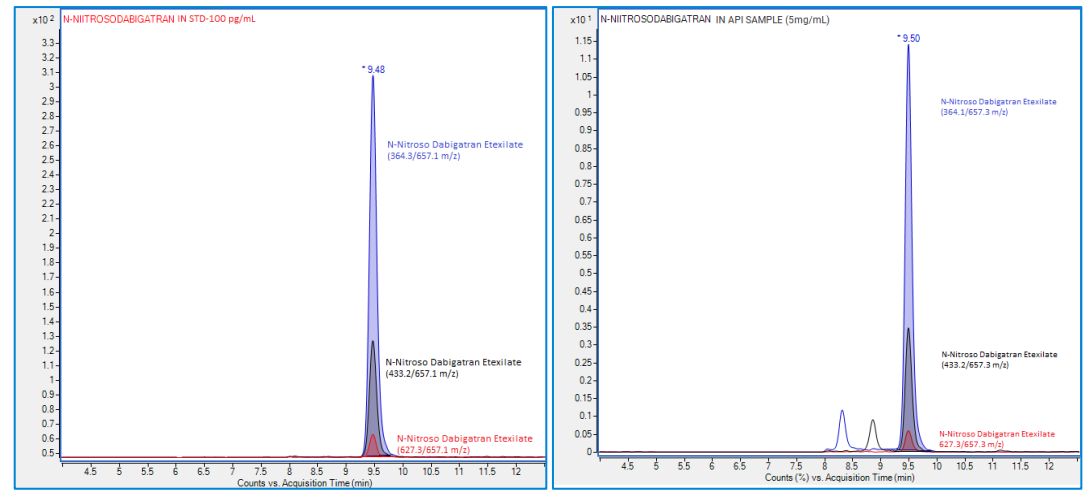

Figure 4. Quantifier and Qualifier ions were overlaid, to evaluate the specificity in matrix and method conditions.

Results and Discussion

Method Precision and Reproducibility:

Method parameters such as method reproducibility were evaluated at specification level concentration (0.1ng/mL; 0.02 ppm spiked in samples with respect to API load 5mg/mL) as described in figure 5.

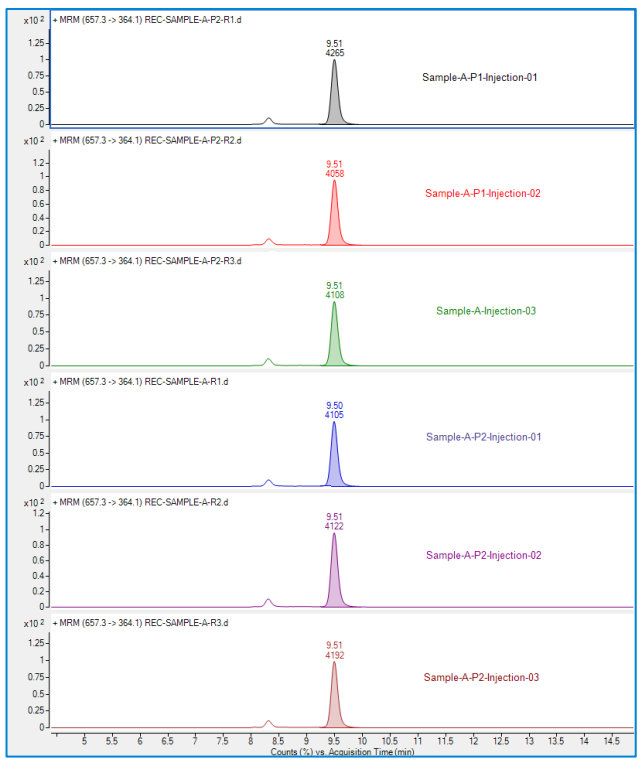

Figure 5. Reproducibility for six consecutive injections of 0.1 ng/mL spiked sample. Were, P1: spike sample set-01 and P2: spike sample set-02. The set were injected three times (N=3). Average in response was consistent and %RSD found to be less than 5%

Method Recovery and Accuracy:

Recovery is performed for ensuring the accuracy and reliability of analytical results. If the recovery is low, it could lead to underestimation of the analyte concentration, while high recovery may indicate contamination or interference.

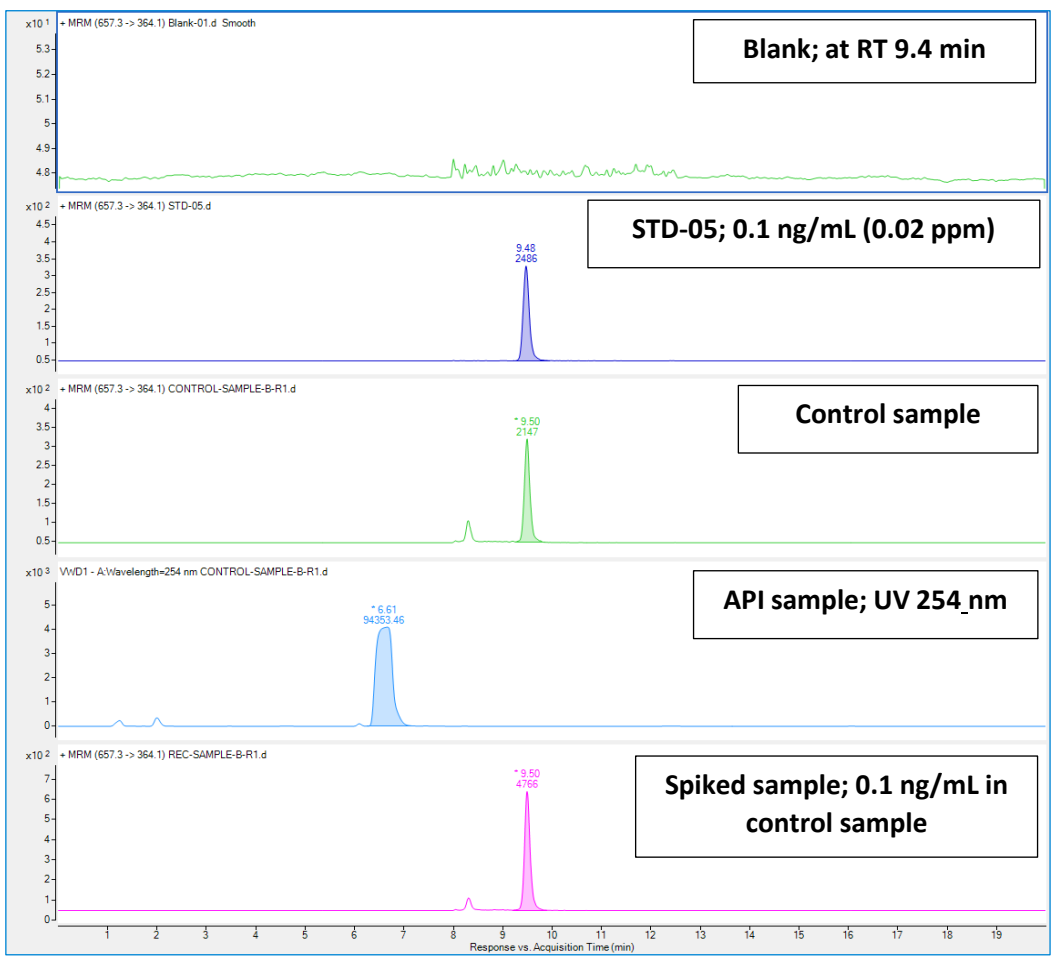

Figure 6. EIC of 364/657 m/z of standard specification limit (0.1 ng/mL; 0.02 ppm), control sample and spiked sample (API load 5 mg/mL)

Recovery is typically expressed as a percentage and calculated using the formula:

Recovery (%)=(Amount of analyte recovered/Amount of analyte spiked)×100%

Replicate (R)	Standard	Control Sample			Spiked Sample		
***n*= 3**	**Specification Limit**	**REF**	**A**	**B**	**REF**	**A**	**B**
R1	2526	1789	1607	2189	4249	4181	4864
R2	2466	1742	1608	2183	4248	4196	4863
R3	2428	1709	1557	2169	4370	4272	4942
Average	2473.33	1746.67	1590.67	2180.33	4289.00	4216.33	4889.67
A = (Average response in sample) –(Average response in control sample)	NA				2542.33	2625.67	2709.30
[A ÷Average response in standard] × 100					**102.78**	**106.15**	**109.54**

Figure 7. Recovery is established in the formulation sample at 0.1 ng/mL (0.02 ppm with respect to API load 5 mg/mL) in three different lots of APIs (sample reference, sample A, and sample B)

Recovery found to be within acceptance window of 90 to 110%

Conclusions

A Highly Sensitive and Robust Multiple Reaction Monitoring (MRM) Method was Developed Utilizing the Agilent 6495C LC/TQ to Quantify N-nitroso Dabigatran Etexilate Impurity in Dabigatran Etexilate API:

- The chromatographic method provided desirable separation between the impurity and the API to avoid interference
- The developed method demonstrated excellent linearity over the range of 0.01 to 1.0 ng/mL (0.002 to 0.02 ppm with respect to API) with R^2 value of 0.999
- The LOD and LOQ values achieved were 6 and 10 pg/mL respectively for targeted NDSRI
- The method provided recovery between 90 and 110%, which is within the acceptance criteria

References

1. Recommended Acceptable Intake Limits for Nitrosamine Drug Substance-Related Impurities (NDSRIs) Guidance for Industry. U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER) August 2023 Pharmacology/Toxicologyhttps://www.fda.gov/drugs/guidance-compliance-regulatory-information/guidances-drugs.
2. 5994-7066E; App note: Low-Level Quantitation of N-Nitroso Dabigatran Etexilate Impurity in Dabigatran Etexilate Mesylate API Using the Agilent 6495C LC/TQ.

https://www.agilent.com/en/promotions/asms

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